EasyBlot Kit User's Manual

- ☐ GTX225856-01 EasyBlot anti-Rabbit IgG Kit (Optimized for Prot A/G)
- ☐ GTX225857-01 EasyBlot anti-Mouse IgG Kit (Optimized for Prot A/G)
- ☐ GTX228909-01 EasyBlot anti-Rat IgG Kit (Optimized for Prot A/G)
- ☐ GTX228910-01 EasyBlot anti-Goat IgG Kit (Optimized for Prot A/G)
- ☐ GTX228911-01 EasyBlot anti-Sheep IgG Kit (Optimized for Prot A/G)

Background:

EasyBlot is an HRP-conjugated second step reagent that specifically reacts with the native, non-reduced form of IgG and does not bind to the reduced, denatured form. Using Easyblot decreases the interference caused by the heavy (~50 kDa) and light chains (~25 kDa) of the IgG used for immunoprecipitation (IP).

The protein A/G beads used in the IP procedure sometimes bind to IgG and produce contaminating signals at approximately 50 kDa and 25 kDa. This kit also includes an EasyBlocker (GTX425858) that can minimize the background caused by protein A/G contamination. GeneTex's EasyBlot kit is simple to use and detects only the target band in your IP experiments, resulting in cleaner western blots.

Note: Suggested dilution is 1:1000. Use 2-5 µg of the IP antibody; do not exceed 5 µg. Completely reduce all IP samples.

Kit contents:

Catalogue number	Product name	Kit contents	
GTX225856-01	EasyBlot anti-Rabbit IgG Kit (Optimized for Prot A/G)	GTX221666-01 EasyBlot anti Rabbit IgG (HRP)	GTX425858 EasyBlocker
GTX225857-01	EasyBlot anti-Mouse IgG Kit (Optimized for Prot A/G)	GTX221667-01 EasyBlot anti Mouse IgG (HRP)	GTX425858 EasyBlocker
GTX228909-01	EasyBlot anti-Rat IgG Kit (Optimized for Prot A/G)	GTX628474-01 EasyBlot anti Rat IgG (HRP)	GTX425858 EasyBlocker
GTX228910-01	EasyBlot anti-Goat IgG Kit (Optimized for Prot A/G)	GTX628547-01 EasyBlot anti Goat IgG (HRP)	GTX425858 EasyBlocker
GTX228911-01	EasyBlot anti-Sheep IgG Kit (Optimized for Prot A/G)	GTX628906-01 EasyBlot anti Sheep IgG (HRP)	GTX425858 EasyBlocker

Preparation of EasyBlocker reagent:

1. Keep the EasyBlocker powder at RT for 10mins before dissolving it.
2. Dissolve the blocking powder in TBST buffer (0.5 g powder in 10 ml TBST buffer). Shaking it for 30~40 mins at RT to make sure that it is dissolved completely.

Note: In order to conserve the EasyBlocker reagent, we recommend diluting the antibody in 2-3 ml of EasyBlocker reagent and incubating the transferred membrane in a sealed bag. Make sure to remove as much air from the bag as possible.

3. We recommend that you reconstitute the EasyBlocker reagent immediately before use.

Protocol:

1. After transferring the IP sample to a PVDF or NC membrane, block the membrane with **5 ml** of **EasyBlocker reagent** for 1 hr at room temperature.
2. Dilute the primary antibody with **2 ml** of **EasyBlocker reagent**. The incubation conditions will depend on the conditions used for the primary antibody.
3. Wash the membrane with TBST buffer 3 times for 5 min each time.
4. Dilute the **EasyBlot anti IgG (HRP)** with **2 ml** of **EasyBlocker reagent** and incubate for 1 hr at room temperature.
5. Wash the membrane with TBST buffer 3 times for 5 min each time.
6. Develop the signal with ECL/ECL+ reagent and detect the signal using either X-ray films or a digital camera.

Note: The recommended dilution ratio for the EasyBlot anti IgG (HRP) is 1:1000. Use 2-5 µg of the IP antibody; do not exceed 5 µg. Completely reduce all IP samples by 100mM DTT.

Recipe:

20X TBS buffer (1 L)	
Tris	48.4 g
NaCl	160 g
Add ddH_2O to 1 L. Mix to dissolve and adjust the pH to 7.0 using concentrated HCl.	

TBST buffer (1 L)	
20X TBS buffer	50 ml
Tween-20	2 ml
Add ddH_2O to 1 L. Adjust pH to 7.0 if necessary.	

Storage instructions:

1. EasyBlocker : store at 4℃.
2. EasyBlot anti IgG (HRP): Aliquot and store as a concentrated solution at -20℃ or below. Avoid multiple freeze-thaw cycles.